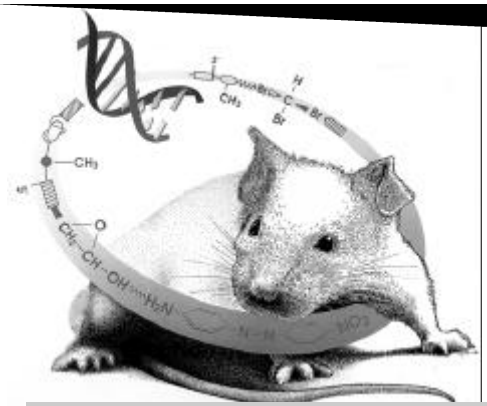

The Tg.AC Workgroup Newsletter

The Tg.AC Workgroup Newsletter is published by The Department of Toxicology and Safety Assessment, Boehringer Ingelheim Pharmaceuticals, Inc. as a means of communication for the HESI's Alternatives to Carcinogenicity Testing Committee.

Letter and article submissions are welcome. Persons interested in contributing to the newsletter should contact:

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On the Inside:

Dermal Carcinogenicity in Transgenic Mice: Effect of Vehicle on Responsiveness of Hemizygous Tg.AC Mice to Phorbol Ester (TPA)

S.M. Furst, R.E. Stoll and J. Mennear
Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT

Since compounds subjected to testing in the Tg.AC model must be solubilized for dermal application, the effect of vehicle on tumor responsiveness must be well-defined to enable interpretation of results. In order to assess the effects of several vehicles on responsiveness of male and female Tg.AC mice to dermally applied TPA, a study was conducted at Boehringer Ingelheim Pharmaceuticals, Inc. beginning in July, 1999. Mice received dermal applications of 2.5 μg of TPA in 200 μl of vehicle three times per week for up to 26 weeks. Vehicles included acetone, ethanol (70 and 100%), methanol (100%), acetone/ethanol (1:1), dimethyl sulfoxide (DMSO), acetone/DMSO (4:1 and 1:1), and acetone/olive oil (4:1). A low dose of 1.25 μg of TPA in 200 μl of DMSO and 100% EtOH as vehicles was also tested to demonstrate the existence of a dose-response relationship in the responsiveness of Tg.AC mice to TPA. When administered in vehicles comprised of acetone, ethanol, methanol or acetone/ethanol TPA caused prompt (7-11 weeks) and maximal tumor responses in both sexes. The presence of DMSO or olive oil reduced the tumorigenic response as well as delayed the time of onset compared to the acetone, ethanol, and methanol vehicles (see charts next page). Also of note was the higher degree of tumor multiplicity in animals treated with TPA in neat DMSO compared to animals treated with TPA in DMSO/acetone mixtures of (1:1) or (1:4). Although a significant effect on response was observed with the DMSO vehicle, a positive response could still be achieved as a result of TPA treatment. Microscopic evaluation of approximately 20 tissues from animals in all dose groups is currently in progress. Investigators wishing to employ solvents other than acetone, ethanol or methanol as vehicles should conduct preliminary experiments to determine the effects of the alternative vehicle on responsiveness to TPA.

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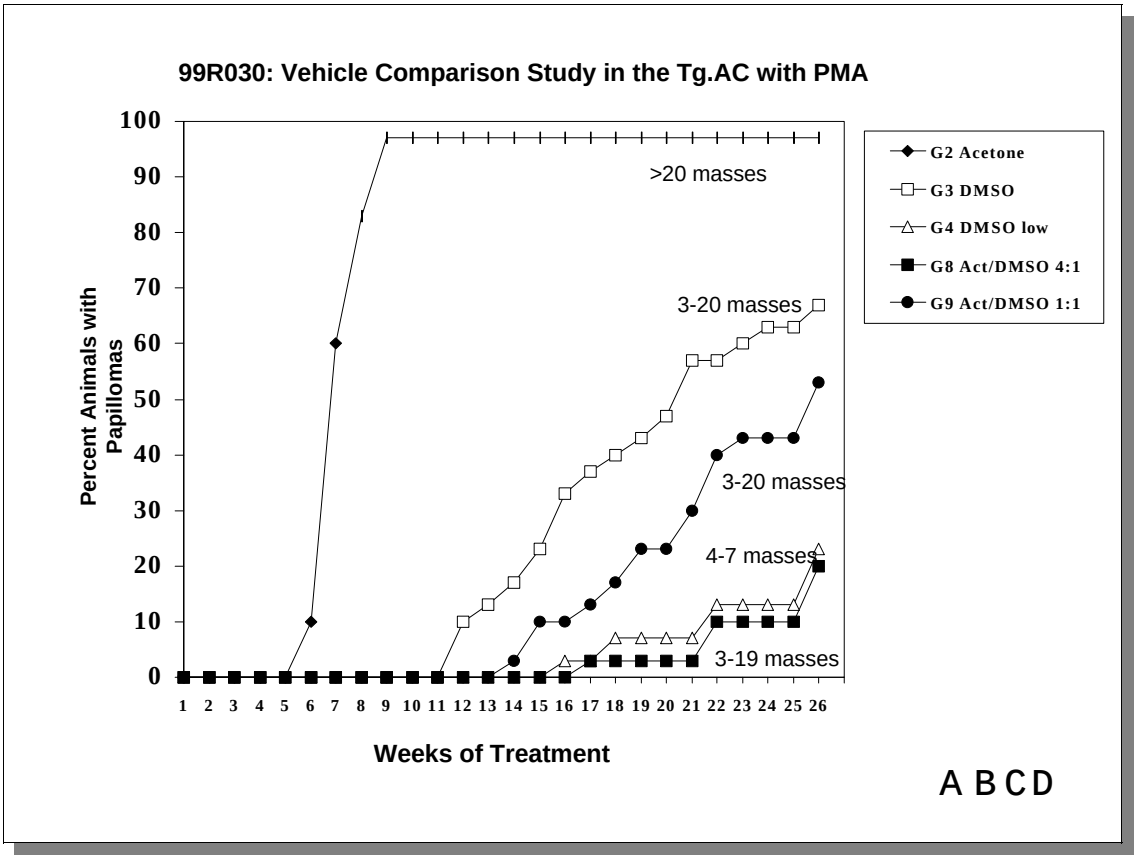

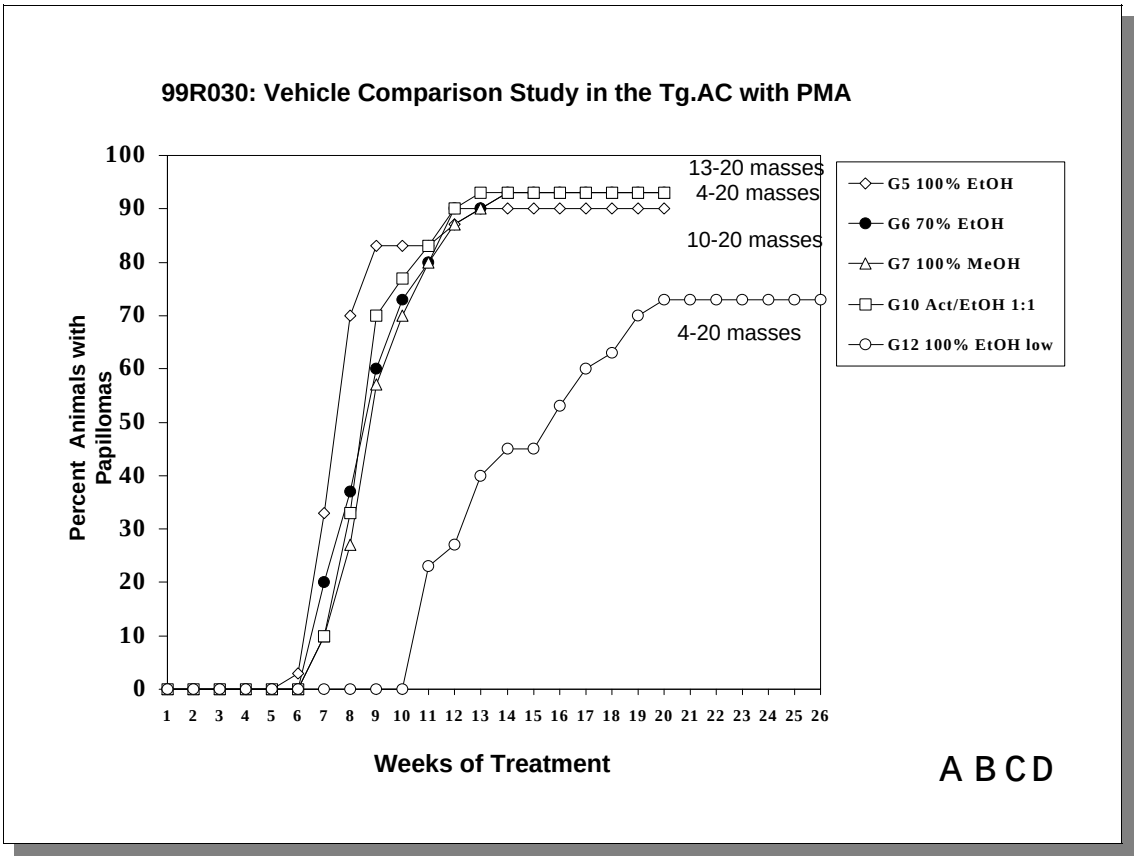

Tg.AC Workgroup Summary
April 19, 2000

J. W. Spaulding
NEHS, RTP, NC

The HESI Alternatives to Carcinogenesis Testing Committee Workshop Organizing Committee held a meeting in Washington, DC at HESI on April 19, 2000 to discuss (1) the agenda for the November 1-3, 2000 ILSI workshop on the Evaluation of Alternative Methods for Carcinogenesis Testing, and (2) the status of the chemical testing studies in the various models.

Most of the meeting, co-chaired by J. S. MacDonald and Denise Robinson, focused on the Draft Agenda for the November meeting. The agenda was fine tuned and several of the program assignments were modified with emphasis placed on adherence to the time-frame of the presentations and keeping a tight rein on the main focus of the meeting.

Several major decisions were:

1. The format for data presentation and summary test results for each test model would be standardized in order to aid the audience in assimilating the information, and

2. The data and summaries of results would be compiled and presented in a final form that would be independent of whether the data for any particular study had been entered into the permanent ACTAR data base. At the time of this meeting, no data for any study had been successfully entered into ACTAR.

An update on the status of study completions for each model (expected dates for draft reports, AWG peer reviews and Final Reports) was presented. The Tg.AC studies (17 chemicals) are most in arrears (compared to all of the other models) with only five draft reports submitted at this date and as many as four draft reports not expected until the end of August.

A joint meeting of the Assay working groups will be held at the ILSI/HESI headquarters in Washington, DC on July 24 and 25. The emphasis will be on an update of study status from the AWGS and data input into ACTAR.

The "Workshop Announcement and Call for Posters" brochure is available and has been sent out. Posters are encouraged and applications (poster abstracts) for posters will be accepted up to July 17, 2000. Poster application forms are included in the brochure.

The brochure is listed under the HESI subheading of the ILSI web site; www.ilsi.org. Otherwise, send a request for a brochure via e–mail to hesi@ilsi.org or by fax to Denise Robinson at (202)-659-3617.

The Tg.AC Assay Work Group will meet on May 10 at NIEHS, RTP, NC.

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Application of the Tg.AC mouse for assessing the protective activity of broccoli juice against TPA and B(a)P-induced papillomas.

Pamela J. Spencer and Bhaskar Gollapudi
The Dow Chemical Company

The Toxicology and Environmental Research & Consulting laboratory of The Dow Chemical Company is continuing its effort to evaluate the utility of the Tg.AC mouse model as a short-term bioassay for the screening of putative carcinogens and anti-carcinogens. We have recently investigated the tumor-modulating activity of broccoli juice on benzo(a)pyrene (B(a)P, complete carcinogen) and TPA (tumor promoter) induced papillomas in Tg.AC mice.

Previous studies conducted in our laboratory and that of others (Gupta *et al*., Reg. Toxicol., 23, 14, 1996; Kassie *et al*., Chemico-Biol. Interact., 102, 1 1996) indicated that extracts and juices prepared from broccoli have genotoxic potential *in vitro*. Hence, we have initially investigated whether broccoli juice itself has any papillomagenic activity in the Tg.AC mouse. No papillomas were observed at the test site of mice dosed dermally (200 µl/mouse, 5/days/week, 26 weeks) with a crude broccoli juice (1:1 ratio in acetone). Mice treated with the phorbol ester TPA (2.5 µg/mouse, 3 days/week for up to 26 weeks) began to develop papillomas at approximately 5 weeks after the start of treatment with 100 % of genotypically responder mice developing papillomas by the end of the 26-week study.

We have subsequently investigated the anti-tumorigenic activity of broccoli juice by dermally administering the juice (200 µl of a 1:1 dilution in acetone/mouse) 1 hour prior to the application of B(a)P (250 µg/mouse) or TPA (2.5 µg/mouse). Five groups of female Tg.AC mice (15/group) were treated with either acetone (vehicle control, 200 µl/mouse), TPA (2.5 µg/mouse), B(a)P (250 µg/mouse), broccoli juice + B(a)P, or broccolic juice + TPA for 13 weeks. The study was terminated after 13 weeks of treatment due to the severity of the lesions in the B(a)P treatment groups.

No papillomas were observed at the dermal test site of mice treated with the vehicle control acetone with the exception of one animal. Mice in all remaining groups began to develop papillomas approximately 3-5 weeks after the start of treatment. Mice treated with B(a)P developed tumors that were grossly and histologically different from tumors in TPA treatment groups. B (a)P-induced tumors were large, ulcerative and histologically identified as primarily squamous cell carcinoma while TPA-induced tumors were small and commonly identified as benign. Treatment with broccoli juice one hour prior to TPA treatment reduced the tumor burden by approximately 50% at the end of the 13-week treatment. In addition to reduced multiplicity, tumor size was qualitatively observed to be smaller in the broccoli juice + TPA treated mice. Pretreatment with broccoli juice did not reduce the tumor burden in B(a)P treated mice. No difference in tumor latency was observed in any of the treatment groups and the number of animals developing papillomas was not different among the treatment groups.

In summary three important conclusions can be drawn from this work. First, the component(s) of broccoli juice responsible for the positive *in vitro* genotoxicity findings did not elicit a tumorigenic response in the Tg.AC mouse possibly

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due to their detoxification. Secondly, broccoli juice exhibited anti-carcinogenic activity against TPA-induced but not B(a)P-induced tumors and these results suggest that the promotional effects of B(a)P are mechanistically different from those of TPA. Finally, the morphological and histological differences between B(a)P and TPA-induced papillomas may suggest the involvement of other mutational events in the etiology and/or progression of B(a)P-induced tumors. These results also provide an initial support for the possibility of using the Tg.AC mouse as a screening tool for assessing anti-carcinogenic potential of test materials.

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Carcinogenic Endpoint Evaluation in Tg.AC Transgenic Mice Given Cyclosporin A Dermally for 26 Weeks

A. M. Hoberman[1], L. G. Lomax[2], and J. H. Wedig[1]. *[1]Primedica Redfield, Redfield, AR and [2]Pathology Consultant, Little Rock, AR.*

The purpose of this study was to assess the potential carcinogenicity of cyclosporin A when applied topically to homozygous Tg.AC mice for 26 weeks. Groups of 15 male and 15 female mice approximately 10 weeks old were given 200 µl/mouse topical doses daily of the following for 26 weeks: acetone vehicle, 2.5 µg of 12-0-tetradecanoly phorbol-13-acetate (positive control), 0.05, 0.4 or 0.8 mg of cyclosporin A dissolved in acetone. Visual observation for papillomas began after 4 weeks and tumor palpation began after 12 weeks. Clinical observations were done daily. Each mouse was necropsied and a complete set of tissues was evaluated histologically. No treatment-related effect was noted on weekly body weight or food consumption. Papillomas were noted grossly and microscopically at the application site in all but two of the positive control mice. There was no significant difference in the survival rates of the three cyclosporin A treated groups when compared to the control group. The number of squamous papillomas at the application site was statistically ($p<0.05$) increased in the females given 0.8 mg cyclosporin A compared to the controls (3/14 vs. 0/14, respectively) but was not statistically increased in the males (2/15 vs. 0/15, respectively). The incidence of all other tumors among cyclosporin A treated animals was comparable to control animals.

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Dermal and Oral Exposure of TCDD in Tg.AC Mice: Dose- and Time-Response Studies

A. P. J. van Birgelen[1], J. D. Johnson[2], A. F. Fuciarelli[3], J. D. Toft[2], M. Hejtmancik[2], J. Mahler[1] and J. R. Bucher[1]. *[1]NIEHS, Research Triangle Park, NC, [2]Battelle Memorial Laboratories, Columbus, OH and [3]Battelle-Toxicology Northwest, Richland, WA.*

Dose-response and exposure route studies were performed with the ubiquitous, bioaccumulative, and carcinogenic agent-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), using the Tg.AC transgenic mouse model in order to evaluate the development of papillomas over time. Female hemizygous Tg.AC mice (20/group) received 0, 5, 17, 36, 76, 121, 166, 355 or 760 ng TCDD/kg in acetone three times a week for 26 weeks by dermal application or 0, 105, 450, or 1250 ng TCDD/kg in corn oil five times a week for 26 weeks by gavage administration. With either route of exposure, TCDD caused neoplastic lesions that were confined to the skin and included squamous cell papillomas and carcinomas. The average number of papillomas per animal and the time to papilloma occurrence were dose-dependent. An increase in the number of papillomas was observed at doses ≥17 ng TCDD/kg (dermal) or 1250 ng TCDD/kg (oral) by study termination. A dose-dependent increase in hepatic and pulmonary cytochrome P4501A activities was observed. TCDD significantly increased hepatic CYP1A1 and CYP1A2 activities in all dose groups (dermal and oral). Pulmonary CYP1A1 activities were significantly increased at doses ≥36 ng TCDD/kg in the dermal study and at all dose levels in the oral study. A remarkable finding in the gavage study was the absence of neoplastic lesions in organs other than the skin.

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Software for Data Entry to the ACT Histopathology Database

The software and supporting users' manual is now available for the database that has been developed to collect histopathology data from the collaborative research program being coordinated by the ILSI Health and Environmental Sciences Institute's Alternatives to Carcinogenicity Testing Committee for the past three years. This database will be used to compile the data from the individual studies in order to facilitate the evaluation and interpretation of the findings from the overall project.

The software for the database is called **ACTAR** ("**ACT** data **A**cquisition and **R**eporting:) and has been developed by Fraunhofer based on the REGINA3 data acquisition program, which is used for the RITA ("Registry of Industrial Toxicology Animal data") and NACAD ("North American Control Animal Database") projects.

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Fraunhofer ITA has set up an ACT member web site (http://www.ita.fhg.de/reni/members/act/index.htm) which will be used in the future to distribute current information on the project among ACT participants. In order to get access to this site, please send your desired user name and password to Mr. Morawietz (actar@ita.fhg.de). You will be notified by e-mail once your accounts have been established.

Loss of Palindromic Symmetry in Tg.AC Mice with a Nonresponder Phenotype

Ronald Honchel[1], Barry A. Rosenzweig[1], Karol L. Thompson[1], Kerry T. Blanchard[2], Sylvia M. Furst[2], Raymond E. Stoll[2], and Frank D. Sistare[1]. *[1]Center for Drug Evaluation and Research, Food & Drug Administration, Laurel, Maryland; [2]Department of Toxicology and Safety Assessment, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut*

The Tg.AC transgenic mouse carries the v-Ha-*ras* oncogene under the control of the ζ-globin promoter and is currently being used in a short-term carcinogenesis assay for safety testing of pharmaceuticals. A subset of hemizygous Tg.AC mice was found to be nonresponsive to the tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA) which characteristically induces skin papillomas in these mice upon repeated dermal application. We have previously shown that both responder and nonresponder hemizygous Tg.AC mice carry about 40 copies of transgene but the nonresponders had lost a 2 kb *Bam*Hl fragment containg ζ-globin promoter sequence. Restriction enzyme and S1 nuclease digestion experiments reported here strongly suggest that the 2 kb *Bam*Hl fragment results from the orientation of two transgenes in an inverted repeat formation. Two subsets of nonresponder Tg.AC mice were identified. Restriction enzyme and S1 nuclease digestion experiments suggest that one nonresponder genotype is produced by a large deletion of one or more near complete copies of transgene sequence and the second genotype is produced by a small deletion near the apex of the "head-to-head" juncture of the inverted repeat. PCR amplification, cloning and sequencing results confirmed the palindromic orientation of transgene in Tg.AC mice. Our results indicate that, despite the presence of multiple copies of transgene in a direct repeat orientation, the loss of perfect symmetry in the palindromic array of transgene sequence results in the complete loss of ability to respond to tumor promoter-induced activation of transgene expression.

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Special Notice

As I will be leaving Boehringer Ingelheim for a new position at Pharmacia, all submissions to the newsletter should be made to R. E. Stoll as indicated on page 1. It was a pleasure working with all who contributed to the newsletter and thank you for helping me make it a success.

—Sylvia Furst

Upcoming Meetings and Events

November 1-3, 2000
"Workshop on the Evaluation of Alternative Methods for Carcinogenicity Testing"

Location:
Lansdowne Resort, Leesburg, VA

For information contact:
Alternatives to Carcinogenicity Testing Workshop
ILSI Health and Environmental Sciences Institute
1126 Sixteenth Street, NW
Washington, DC 20036-4810
Fax: (202) 659-3617
Email: meetings@ilsi.org (Please refer to Alternatives to Carcinogenicity Testing Workshop)
Visit ILSI's website: http://www.ilsi.org

November 9-11, 2000
"Experimental Skin Carcinogenesis" Fourth International Conference

Location:
The University of Arizona, Arizona Cancer Center, Tucson

For information, contact:
G. Tim Bowden
The University of Arizona
PO Box 245024
Tucson, Arizona 85724-5024
FAX: (520)-626-4979
Email: tbowden@AZCC.Arizona.edu

November 29-December 3, 2000
"Mouse Models of Cancer"

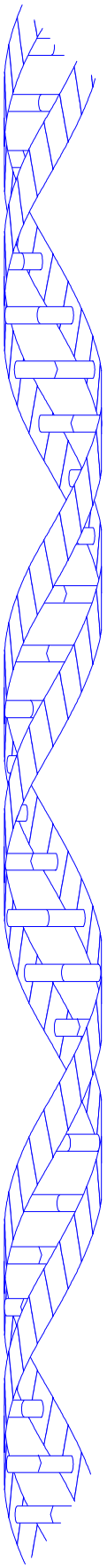
Chair persons:
Tyler Jacks, Cambridge, MA
Ronald A. DePinho, Boston, MA

Location:
Hyatt Regency, La Jolla, La Jolla, CA

February 25-March 1, 2001
"Cancer Susceptibility Genes and Molecular Carcinogenesis"

Chairpersons:
Allan Balmain, San Francisco, CA
Bruce A.J. Ponder, Cambridge, England

Location:
Hyatt Regency Lake Tahoe
Incline Village, NV



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